

FURTHER EVIDENCE FOR CHLORPYRIFOS TOLERANCE AND
PARTIAL RESISTANCE BY THE JAPANESE BEETLE
(COLEOPTERA: SCARABAEIDAE)

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Abstract.—Dosage-mortality responses of third-instar grubs of the Japanese beetle, *Popillia japonica* Newman, showed that a population from Fairfield (Conn.), when compared at the LD₉₅, is ca. 42× as tolerant to chlorpyrifos as those from Adelphia (central N.J.; a standard susceptible population). Moreover, the 95% conf. intervals of the LD₅₀ and LD₉₅ of the Fairfield population did not overlap with those of the Adelphia population. Another population from Rivervale (northern N.J.) also showed 6.8-fold greater tolerance to chlorpyrifos, however, the conf. intervals of its LDs did overlap with those of the susceptible strain. The Fairfield population was characterized as “partially-resistant,” and that from Rivervale as “tolerant,” to chlorpyrifos.

The grubs of the Japanese beetle, *Popillia japonica* Newman, are highly destructive to turfgrass in several states east of Mississippi River (Fleming 1972). Chlordane was first used for grub control in 1947 (Fleming 1950), and since then for nearly twenty-five years chlordane and other cyclodiene insecticides, e.g., dieldrin and heptachlor, afforded good protection against Japanese beetles. Beetle resistance to cyclodienes was first reported in 1973 from Ohio (Niemczyk and Lawrence 1973), New York (Tashiro and Neuhauser 1973), and Pennsylvania (Anon. 1973). Subsequently, cyclodiene resistance among beetle populations of Connecticut (Dunbar and Beard 1975), and New Jersey (Ahmad and Das 1978) also were documented. Furthermore, because of environmental concerns the use of highly persistent cyclodiene insecticides has been restricted by the Environmental Protection Agency. Consequently, nonpersistent organophosphorus insecticides, chlorpyrifos, diazinon and trichlorfon have replaced cyclodienes for control of the Japanese beetle.

In 1977, a population of the Japanese beetle from New Jersey (Rivervale) was suspected of increased tolerance to chlorpyrifos (Ahmad and Das 1978). The following year, the greater tolerance of chlorpyrifos by the Rivervale beetle was confirmed by more extensive tests, using susceptible beetles for comparison (Ng and Ahmad 1979). We now provide additional evidence for a much greater chlorpyrifos tolerance in a Connecticut population of the beetle, compared to the New Jersey insects.

Materials and Methods

Third-instar grubs of the Japanese beetle from Fairfield, Conn. (Brooklawn Country Club golf course) were supplied by A. M. Radko (U.S. Golf Assocn., Far Hills, N.J.) on October 1, 1979. The grubs were held in the laboratory for ca. 48 hr period and then tested for susceptibility to chlorpyrifos. The procedure and laboratory conditions for the maintenance of the grubs were exactly the same as reported earlier (Ahmad and Das 1978). For comparisons of susceptibility levels, grubs were collected from a chlorpyrifos-tolerant population in Rivervale (northern N.J.; Country Club golf course), and a standard susceptible population from Adelphia (central N.J.; Soils and Crops Res. Ctr., Rutgers Agric. Exp. Stn.) on October 7 and 13, respectively. Grubs from these two populations also were held in the laboratory for ca. 48 hr prior to screening for chlorpyrifos susceptibility.

The grubs were treated topically with one-microliter drops of chlorpyrifos solutions, in 5 replicates, 10 grubs per replicate. Each test was repeated twice and the data were pooled for analyses. Solvent controls were included. The composition of the insecticide solvent, and the technique for the bioassay including that for grub rearing, were as reported earlier in detail (Ahmad and Das 1978). Mortality was recorded on 8th post-treatment day since the duration of the acute toxicity of chlorpyrifos in the Japanese beetle is 7 days (loc. cit.). Mortality data were corrected for deaths in the control group by Abbott's formula, and subjected to probit analysis according to Finney (1964). The regression coefficients (slopes) for the log dose-probit (*ldp*) lines of the 3 populations also were analyzed for statistical difference.

Results and Discussion

The dosage-mortality responses of the Japanese beetle grubs from Adelphia (N.J.), Rivervale (N.J.) and Fairfield (Conn.) are shown in Fig. 1. The LD values and their 95% confidence intervals are presented in Table 1. The Rivervale population shows a 6.8-fold greater tolerance to chlorpyrifos at the LD₉₅ level to that of the susceptible population. Tests with adult beetles also have shown the Rivervale population to be more tolerant to chlorpyrifos than the Adelphia insects (Ng and Ahmad 1979). A 4.3× greater tolerance was discerned with a significant difference between the regression coefficients (*b*) of the *ldp* lines of the two populations (loc. cit.). In the present investigation involving third-instar grubs, the regression coefficients of the *ldp* lines of the Rivervale (*b* = 0.96) and Adelphia (*b* = 2.43) populations also were found to be significantly different [*t* (*df*, 5) = 11.315, *P* < 0.05]. Thus our earlier conclusion that there were more chlorpyrifos-tolerant individuals in the Rivervale population than in the Adelphia population (Ng and Ahmad 1979) was reaffirmed. The data on chlorpyrifos tolerance of the

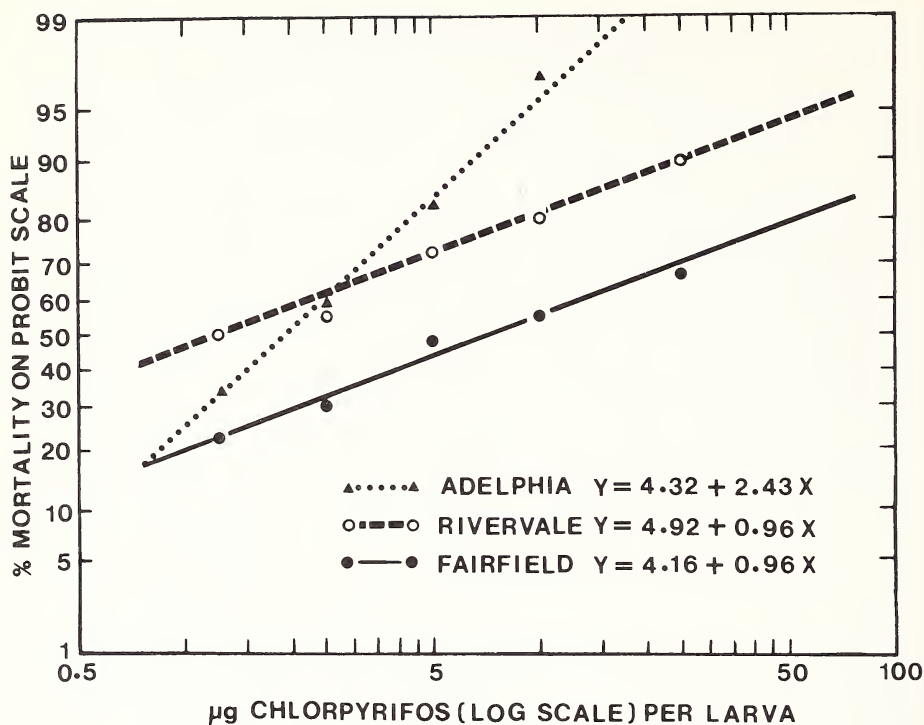


Fig. 1. Dosage-mortality responses of third-instar grubs of the Japanese beetles from Fairfield (Conn.), Rivervale (northern N.J.), and Adelphia (central N.J.); 1979.

Rivervale beetles are typical of a "tolerant" insect population showing incipient resistance to an insecticide (Busvine 1971).

Table 1 also indicates that by comparison to the Rivervale population, grubs from Fairfield are exceedingly tolerant to chlorpyrifos. At the LD_{95} level the Fairfield insects are ca. $42\times$ as tolerant as the susceptible Adelphia population, and ca. $6\times$ more tolerant than the Rivervale population. As can be expected the regression coefficient of the ldp line of the Fairfield ($b = 0.96$) grubs was significantly different to that of the Adelphia ($b = 2.43$) population [t (df , 5) = 10.145; $P < 0.05$]. Moreover the 95% C.I.s of the LD_{50} and LD_{95} of the Fairfield population showed no overlap to the corresponding C.I.s of the Adelphia insects. Taken together, these data clearly show that the selection towards more chlorpyrifos-tolerant individuals in the Fairfield population has progressed further than in the chlorpyrifos-tolerant Rivervale population.

According to Dyte and Blackman (1967) if a population of insects is re-

Table 1. Comparison of chlorpyrifos susceptibility of third-instar grubs of the Japanese beetle from Fairfield (Conn.), Rivervale (northern N.J.), and Adelphia (central N.J.); 1979.

Population ^a	LD ₅₀ ^b	LD ₉₅ ^b	LD ₉₅ ratio
Adelphia	1.90 (1.45–2.56)	9.17 (5.50–15.42)	1
Rivervale	1.20 (0.50–2.84)	62.45 (13.29–293.50)	6.8
Fairfield	7.50 (4.42–12.58)	388.06 (45.29–3,324.30)	42.2

^a Mean weight of the grubs (N = 10) were 208 mg (±25 SD) for Adelphia, 196 mg (±39 SD) for Rivervale, and 185 mg (±24 SD) for Fairfield populations.

^b Confidence intervals (*P* = 0.05) in parentheses.

sistant to an insecticide, the LD_{99,99} of the resistant strain must be significantly greater than that of the susceptible strain. From the dosage-mortality data in Table 1, we computed the lower C.I. for the LD_{99,99} of the Fairfield grubs to be 579 µg/grub; this did not overlap with the upper C.I. of the LD_{99,99} of the Adelphia population, 221 µg/grub. Thus the Fairfield population appeared to meet the requirement for characterization as a resistant population. On the other hand, as mentioned above the regression coefficient of the *ldp* line for the Fairfield population was considerably flatter than that of the Adelphia population. A flat *ldp* line is often indicative of the genetic variability in insecticide susceptibility of a population. A truly resistant population typically depicts steep slope as is usually the case with a homogeneous susceptible population (Busvine 1971). Therefore, it would be premature to assume that the entire population from Fairfield is resistant to chlorpyrifos, but rather it is an example of a partially-resistant field population, with more resistant insects in the population than susceptible ones. The Fairfield population obviously has the potential for rapidly becoming a highly resistant field population under further selection against chlorpyrifos.

Selection of a population against an organophosphorus insecticide often confers resistance to other organophosphorus compounds (Perry and Agosin 1974). There is some indication that Japanese beetles may develop resistance to organophosphorus compounds other than chlorpyrifos. For example, trichlorfon and diazinon were as ineffective as chlorpyrifos during 1979 against the grubs of the Fairfield population (A. M. Radko, pers. commun., 1979). On the other hand, trichlorfon afforded good protection against the Rivervale grubs that are substantially more susceptible to chlorpyrifos than the Fairfield population (W. Gaydosh, pers. commun., 1979).

In a previous report (Ng and Ahmad 1979), we had also shown the greater inherent tolerance ($2.1\times$) by the Rivervale beetles to bendiocarb, a potential carbamate insecticide for grub control. Although we did not test bendiocarb against the Fairfield strain, the possibility exists, that with time, the Japanese beetle populations also may become resistant to carbamate insecticides. This would not be surprising since the mode of action and detoxication mechanisms associated with tolerance and resistance are common to both organophosphorus and carbamate insecticides (Perry and Agosin 1974; Plapp 1976).

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